IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Denise L. FAUSTMAN		
Serial No.:	09/913,664	ART UNIT:	1651
Filed:	August 17, 2001	EXAMINER:	V. Afremova
Entitled:	METHOD FOR INHIBITING TRANSPLANT REJECTION		

Attorney Docket No.: DLF-002.1P US

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TRANSMITTAL LETTER AND REQUEST FOR EXTENSION OF TIME

Sir:

Transmitted herewith are: [X] an Appeal Brief Under 37 C.F.R. §41.37; [X] a check (no. 5475) in the amount of **$1,210.00** in payment of the fees under 37 C.F.R. § 1.17(a)(5) & 37 C.F.R. § 41.20(b)(2); and [X] a return postcard.

FEE FOR ADDITIONAL CLAIMS

[X] A fee for additional claims is not required.

[ ] A fee for additional claims is required. The additional fee has been calculated as shown below:

	TOTAL CLAIMS	HIGHEST NUMBER PREVIOUSLY PAID FOR	NUMBER OF EXCESS CLAIMS	RATE	FEES DUE
TOTAL CLAIMS			0	× $9.00	= 0.00
INDEPENDENT			0	× $44.00	= 0.00
FIRST INTRODUCTION OF MULT. DEPENDENT CLAIM +$150.00					= 0.00
TOTAL FEES DUE					= **00.00**

[X] Small entity status has already been established for Applicant(s) in this case.

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PETITION FOR EXTENSION OF TIME

[X] Extension is requested under 37 CFR 1.136(a), and the following extension fee is applicable for the paper(s) filed herewith: [ ] $55.00 for response within first month pursuant to 37 CFR 1.17(a)(1);
[ ] $215.00 for response within second month pursuant to 37 CFR 1.17(a)(2);
[ ] $490.00 for response within third month pursuant to 37 CFR 1.17(a)(3);
[ ] $765.00 for response within fourth month pursuant to 37 CFR 1.17(a)(4);
[x] $1040.00 for response within fifth month pursuant to 37 CFR 1.17(a)(5).

[X] Total amount of payment in connection with the paper(s) transmitted herewith is **$ 1,210.00**. A check in in that amount accompanies this paper. (check no. 5475)

[X] The Commissioner is hereby authorized to charge payment of any additional fees required in connection with the paper(s) transmitted herewith, or to credit any overpayment of same, to Deposit Account No. 50-0268 . A duplicate copy of this transmittal letter is transmitted herewith.

Respectfully submitted,

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CERTIFICATE OF MAILING

I hereby certify that the correspondence listed above is being deposited with the United States Postal Service as First Class Mail, postage prepaid, in an envelope addressed to: Mail Stop Appeal Brief – Patents, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date indicated below.

Oct. 25, 2004
Date

David G. O'Brien

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application of:	Denise L. Faustman	**ON APPEAL**
Serial No.:	09/913,664	ART UNIT: 1651
Filed:	August 17, 2001	EXAMINER: V. Afremova
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Atty. Docket No.: DLF-002.1P US

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

BRIEF ON APPEAL

Sir:

Pursuant to 37 C.F.R. §41.37, Appellants submit this Brief on Appeal, setting forth the basis of their appeal from the final Office Action, mailed September 24, 2003 finally rejecting Claims 1-14 and 16-23 of the above-identified patent application.

Notice of Appeal pursuant to former 37 C.F.R. §1.191 was timely filed on March 24, 2004.

This Brief is accompanied by the small entity filing fee under 37 C.F.R. §41.20(b)(2) and a petition for extension of time under 37 C.F.R. §1.136(a) with the appropriate fee under 37 C.F.R. §1.17(a)(5). The Commissioner is hereby authorized to charge any additional fees required in connection with the filing of this Brief to PTO Deposit Account No. 50-0268.

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TABLE OF CONTENTS

REAL PARTY IN INTEREST

Appellant hereby identifies Denise L. Faustman, M.D., Ph.D., as the owner of the invention disclosed in the present application.

RELATED APPEALS AND INTERFERENCES

No related appeals or interference proceedings are known to Appellant or Appellant's legal representatives that are related to the present appeal or that will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

STATUS OF CLAIMS

The present application was filed with Claims 1-37. The present status of all claims is shown in the following table.

Claim No.	Claim Status	Status on Appeal
1	rejected	appealed
2	rejected	appealed
3	rejected	appealed
4	rejected	appealed
5	rejected	appealed
6	rejected	appealed
7	rejected	appealed
8	rejected	appealed
9	rejected	appealed
10	rejected	appealed
11	rejected	appealed
12	rejected	appealed
13	rejected	appealed
14	rejected	appealed
15	canceled	
16	rejected	appealed

17	rejected	appealed
18	rejected	appealed
19	rejected	appealed
20	rejected	appealed
21	rejected	appealed
22	rejected	appealed
23	rejected	appealed
24	canceled	
25	canceled	
26	canceled	
27	canceled	
28	canceled	
29	canceled	
30	canceled	
31	canceled	
32	canceled	
33	canceled	
34	canceled	
35	canceled	
36	canceled	
37	canceled	

The appealed Claims 1-14 and 16-23 appear in the attached Claims Appendix (Tab A).

STATUS OF AMENDMENTS

All of Appellants' amendments have been entered.

SUMMARY OF CLAIMED SUBJECT MATTER

The present invention is directed to a method for inhibiting rejection of transplanted donor tissue by treating **viable** mammalian allogeneic and xenogeneic transplant tissue, for example, by enzymatic treatment, in such a manner as to **temporarily ablate** MHC Class I antigen complexes from the surface of the transplant tissue, then transplanting the treated donor tissue into a host before reappearance of MHC Class I antigen complexes occurs on said donor tissue. (See, e.g., page 3, line 30, to page 4, line 2, of the specification and Claim 1.) Ablation of MHC Class I antigen complexes on the donor tissue prevents the immediate immune recognition and attack by cytolytic T cells (CTLs) of the host (such recognition and attack being MHC Class I antigen complex-mediated processes); and thereafter, continued expression by the **viable** transplant tissue of MHC Class I molecules results in the gradual reappearance of MHC Class I antigen complexes on the surface of the transplant tissue cells. (That is, the ablation of the MHC Class I antigen complexes from the donor tissue is **temporary**, see page 5, line 32, to page 6, line 2.) The gradual re-expression and re-presentation at the surface of the donor cells of MHC Class I antigen complexes provides the normal mechanism for educating the host's immune system to identify the new (donor) tissue as "self" and to cause deletion of the subpopulation of natural CTLs capable of recognizing and rejecting the donor tissue. (See, e.g., page 6, lines 6-12, and Example 2, on pages 9-10 of the application.)

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The issues for consideration in the present appeal are:

I. Whether the methods recited in appealed Claims 1-3 and 5-8 are anticipated under 35 U.S.C. §102(b) by Oliver et al., U.S. Pat. No. 4,399,123 ("Oliver I").

II. Whether the methods recited in appealed Claims 1-3, 5-7, 9, and 12 are anticipated under 35 U.S.C. §102(b) by Oliver et al., U.S. Pat. No. 5,397,353 ("Oliver II").

III. Whether the methods recited in appealed Claims 1-9, 12-14, and 16-23 would, at the time of Appellant's invention, have been obvious under 35 U.S.C. §103(a) to a person of ordinary skill in the art of immunology in view of the combined teachings of Oliver I and Oliver II, taken with Galati et al., *Cytometry*, 27: 77-83 (1997) ("Galati").

IV. Whether the methods recited in appealed Claims 10 and 11 would, at the time of Appellant's invention, have been obvious under 35 U.S.C. §103(a) to a person of ordinary skill in the art of immunology in view of the combined teachings of Oliver I and Oliver II, taken with Galati and further in view of Stone et al., *Transplantation*, 65: 1577-1583 (1998) ("Stone").

ARGUMENTS

The final rejection of the appealed claims is in error for the following reasons:

1. The Oliver I reference cited by the Examiner does not disclose a method for inhibiting rejection of viable donor tissue by a host mammal (1) by treating the viable donor tissue with an enzyme such as papain to temporarily remove (ablate) MHC Class I surface antigens prior to transplantation, and (2) by maintaining the viable donor tissue in the host after transplantation such that MHC Class I surface antigens are re-expressed on the surface of said donor tissue. The Oliver I reference cannot teach part (1) of Appellant's invention because that reference only discloses the use of non-viable fibrous tissue for use in transplantation; and the Oliver I reference cannot teach part (2) of Appellant's invention because the non-viable tissue utilized according to that reference is incapable of regenerating MHC Class I antigen complexes for induction of tolerance to the transplanted tissue. Accordingly, since the Oliver I reference is incapable of teaching critical recited elements of Appellant's appealed claims, the Oliver I reference cannot be considered to anticipate appealed Claims 1-3 and 5-8 under 35 U.S.C. §102(b).

2. The Oliver II reference cited by the Examiner does not disclose a method for inhibiting rejection of viable donor tissue by a host mammal (1) by treating the viable

donor tissue with an enzyme such as papain to temporarily remove (ablate) MHC Class I surface antigens prior to transplantation, and (2) by maintaining the viable donor tissue in the host after transplantation such that MHC Class I surface antigens are re-expressed on the surface of said donor tissue. The Oliver II reference cannot teach part (1) of Appellant's invention because that reference only discloses the use of non-viable fibrous tissue for use in transplantation; and the Oliver II reference cannot teach part (2) of Appellant's invention because the non-viable tissue utilized according to that reference is incapable of regenerating MHC Class I antigen complexes for induction of tolerance to the transplanted tissue. Accordingly, since the Oliver II reference is incapable of teaching critical recited elements of Appellant's appealed claims, the Oliver II reference cannot be considered to anticipate appealed Claims 1-3, 5-7, 9, and 12 under 35 U.S.C. §102(b).

3. The combination of Oliver I and Oliver II in view of Galati utterly fails to present a suggestion of the method of Appellant's invention as defined by the claims on appeal. The reference combination, first of all, does not contain a teaching relevant to the transplantation of viable cells. This is because both of the Oliver I and II references relate ONLY to the preparation of non-viable fibrous tissue for use in transplantation, and Galati does not relate to transplantation at all, merely disclosing a method for quantitating the amount of MHC Class I molecules on the surface of living cells involving cleavage of the MHC Class I antigen complexes followed by affinity purification, acid isolation of β_2-microglobulin and correlation of the amount of β_2-microglobulin with level of MHC Class I expression.

Second of all, the reference combination does not contain any teaching to preserve the ability of donor tissue being prepared for transplant to re-express MHC Class I antigen complexes after transplantation, as required by Appellant's claims. This is because the Oliver I and II references teach only the removal of antigenic structures from transplant tissue and follow such removal with treatments guaranteed to render the donor tissue incapable of re-expressing any further MHC Class I antigen complexes after transplantation, and the Galati reference relates only to the collection and quantitation of

removed MHC Class I complex components, making no mention of any use for the cells from which the MHC Class I antigen complexes are removed.

4. Finally, the combination of Oliver I and Oliver II taken with Galati and further in view of Stone utterly fails to present a suggestion of the method of Appellant's invention as defined in appealed Claims 10 and 11. First of all, the reference combination does not contain a teaching relevant to the transplantation of viable cells: Oliver I, Oliver II, and Stone all teach pre-transplant treatments rendering the donor tissues non-viable; Galati relates to *in vitro* quantitation methods and not transplantation. Second of all, the reference combination does not contain any teaching to preserve the ability of viable donor tissue intended for transplant to re-express MHC Class I antigen complexes after transplantation: Oliver I, Oliver II, and Stone teach the permanent removal of antigenic structures from tissue rendered incapable of re-expressing any antigenic epitopes post-transplant; Galati discusses only the collection and quantitation of antigen complexes removed from cells and makes no mention of any use for the cells from which such antigen complexes are removed.

The following discussion provides the factual and legal basis for the reasons set forth above for finding error in the final rejection of Appellant's claims.

Introductory: The art cited to reject Appellant's claims is not relevant to the invention as claimed

Generally, the principle of transplantation of live tissue from a donor into a host is known (*see*, paragraph bridging pages 2-3 of Appellant's application), yet the Examiner has resorted to publications relating to the transplantation of lifeless collagenous tissues (Oliver I and II) and cartilage (Stone), and relating to *in vitro* quantitation methods (Galati), in order to argue that the claimed method of Appellant's invention was known or would have been obvious at the time of invention. Because none of the cited references or any combination of their teachings can guide a person skilled in this art to a method for successfully inhibiting rejection of living transplant tissues by a host, the cited references and combinations proposed by the Examiner by definition cannot meet or suggest the

invention defined in the appealed claims within the meaning of 35 U.S.C. §102(b) or §103(a). Accordingly, reversal of the final rejections based on the cited art is respectfully believed to be in order.

I. The Oliver I reference does not anticipate the invention of Claims 1-3 and 5-8 under 35 U.S.C. §102(b)

For anticipation under 35 U.S.C. §102 by a printed publication, that publication must teach each and every element or aspect of the claimed invention. As stated in MPEP §2131:

> **"TO ANTICIPATE A CLAIM, THE REFERENCE MUST TEACH EVERY ELEMENT OF THE CLAIM**
> 'A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.' *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). 'The identical invention must be shown in as complete detail as is contained in the . . . claim.' *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989)." (emphasis in original)

There are clearly elements set forth in Appellant's claims which are not taught in the Oliver I publication, hence the rejection for anticipation is in error. For example, considering appealed Claim 1, the claim elements and the teachings of the reference are contrasted below:

Elements of Appealed Claim 1, reciting a method for inhibiting rejection by a host mammal of donor tissue from another mammal, the method comprising:	Contrast with teaching of Oliver I
(a) treating **viable** donor tissue with an enzyme effective for **temporarily** ablating MHC Class I antigens from said donor tissue,	Oliver I teaches the preparation for transplantation of nonantigenic fibrous tissue (col. 2, lines 9-29), by a treatment which renders the tissue non-viable: *See* ▪ references to sterilization of the tissue at col. 3 (ln. 47), col. 4 (ln. 53), col. 5 (ln. 23-25), ▪ teaching of formaldehyde or glutaraldehyde treatment, preferably for 28 days or longer at col. 5 (ln. 11-20), and Examples 1-6 ▪ treatment with sodium azide for 28 days, Examples 1-4 and 6
(b) transplanting said treated, viable donor tissue into said host mammal before MHC Class I antigens are re-expressed on the surface of said donor tissue, and	The inert fibrous tissue prepared according to Oliver I is not viable and is incapable of regenerating MHC Class I antigens. No mention is made in Oliver I of preserving the ability of the donor tissue to re-express the antigenic structures removed by pre-transplantation enzyme treatment.
(c) maintaining said viable donor tissue in said host.	The transplanted non-viable, fibrous donor tissue becomes colonized by living host cells and vascularized.

The direct recitation in Appellant's Claim 1 of using "viable" donor tissue thus immediately renders the teachings of Oliver I irrelevant and non-anticipatory to the present invention. So does the direct recitation in Claim 1 of treating the donor tissue to "temporarily ablate" MHC Class I antigens, which preserves the ability of the donor tissues to re-express the MHC Class I antigens post-transplant. Oliver I cannot meet this feature of Appellant's claims, because all references to antigenic structures in Oliver I relate to their removal from the transplant tissue and none relate to reassertion of such structures. Since Oliver I does not meet recited features in Appellant's Claim 1, the rejection of Claim 1 as anticipated by Oliver I is in error and should be reversed.

Since appealed Claims 2-3 and 5-8 likewise incorporate the recitations of viable transplant tissue and the retained ability of the donor tissue for expressing the MHC Class I antigen complexes that were ablated prior to transplant, those claims also cannot be anticipated by Oliver I. Accordingly, their rejection as anticipated by Oliver I should likewise be reversed.

Elements of appealed Claims 5-7 additionally not met by Oliver I

In addition to the foregoing, appealed Claims 5-7 are not anticipated by Oliver I because they specifically recite transplant tissues not contemplated by Oliver I. Claim 5 specifies that the donor tissue comprises blood cells, neurons, hepatocytes, cardiac cells, genetically modified cells, skin cells, precursor cells, endothelial cells, fibroblasts, myoblasts, islets of Langerhans cells, or bone marrow cells; Claim 6 specifies that the donor tissue is an organ or part of an organ; Claim 7 specifies that the organ is selected from the group consisting of skin, kidney, heart, pancreas, brain, and liver.

Oliver I only discloses the isolation, sterilization, and transplantation of fibrous tissue, i.e., dermis, ligament, tendon, areolar tissue, basement membrane or dura mater from which "all cellular elements . . . are removed." *See*, col. 1, lines 56-65. Thus, the tissues specified in Claims 5-7 also are claim elements not anticipated by Oliver I.

The Examiner's Arguments

The Examiner's answer to Appellant's point that the Oliver I reference relates to the use of non-viable tissue (as opposed to the viable tissue recited in Appellant's claims) was to argue that the treatment taught by Oliver I was not taught to be lethal to the tissue. In other words, the Examiner challenged what Appellant asserted would be conveyed to a person skilled in the art, namely, that the Oliver I teaching was *designed* to eliminate viable tissue from the fibrous tissue selected in Oliver I for transplant. In the final Office Action, the Examiner argued:

> "However, upon review of the teaching of the [Oliver I reference] is it established that the [Oliver I reference] *does not disclose* that the donor tissue is rendered dead or non-

> viable as the result of treatment before transplantation. [The Oliver I reference] clearly teaches that sodium azide is used as 'bactericide' . . . which is reasonably expected to achieve elimination of a possible pathogen transmission during transplantation *rather than to render the donor tissue dead or non-viable*". (See, final Office Action, page 4.) (emphasis added)

The stated intention of Oliver I to kill bacteria (bactericide) does not change the FACT that all viable donor cells associated with the selected fibrous transplant tissue are killed by the preparatory treatment as well. It is improper for the Examiner to ignore this fact of biology merely because her chosen reference is silent about it. Appellant asserts that although the Examiner may seek to deny a biological reality, a person skilled in the art would immediately understand that Oliver I teaches selection of transplant tissue for its mechanical features that can be rendered free of viable cells, and that the pre-transplant treatments that are directly taught by Oliver I assure the non-viability of the fibrous tissue.

Moreover, in view of the Examiner's position, Appellant provided a declaration under 37 C.F.R. §1.132 in order to demonstrate to the Examiner that treatment of viable cells according to the Oliver I patent, e.g., in 0.5mg/ml sodium azide for 28 days, would, without question, result in 100% elimination of viable cells. The declaration of the inventor, Denise L. Faustman, M.D., Ph.D., pursuant to 37 C.F.R. §1.132 was entered into the record in the Advisory Action mailed August 27, 2004; a copy is attached in the Evidence Appendix (Tab B).

The declaration of Dr. Faustman includes a set of experiments demonstrating the treatment of various human- and murine-derived eukaryotic cells with varying concentrations of sodium azide ranging from 0 to 0.5mg/ml (the latter, and highest concentration tested, 0.5mg/ml, being the concentration disclosed for treating tissues prior to transplantation in the Oliver I patent). Specifically, Dr. Faustman treated murine liver cells, kidney cells, and splenocytes, and also human peripheral blood lymphocytes (PBLs), with 0, 0.001, 0.002, 0.01, 0.02, 0.10, and 0.5mg/ml sodium azide. Results of these treatments are shown in Tables 1A, 1B, and 1C included in the declaration. As seen

in Tables 1A, 1B, and 1C, 100% of the various cells treated with sodium azide concentrations as low as 0.001mg/ml were non-viable within three days.

The declaration of Dr. Faustman also includes a demonstration of the effect of formaldehyde treatment on the viability of transplant tissue, in accordance with Example 5 of Oliver I. As shown in Tables 2A, 2B, and 2C of this declaration, a five-minute treatment with 0.1% formaldehyde resulted in 100% death of all cells within three days. By contrast, the Oliver I reference teaches formaldehyde treatment at a concentration of 0.01% to 10% for "a period of treatment for up to 28 days or even longer is preferred." (*See*, Oliver I at col. 5, lines 19-20.) Example 5 of Oliver I shows 0.1%, 1% and 5% formaldehyde treatment for 21 days.

These experiments graphically illustrate what is known, as a matter of law, by the hypothetical person of ordinary skill in the art, i.e., that the pre-transplant treatments of Oliver I render the donor tissue non-viable (regardless of whether such non-viability is discussed *in haec verba* in the Oliver I reference or not). This being the reality of the Oliver I teachings, the Oliver I patent cannot be considered to teach Appellant's invention, which calls for transplantation of viable tissue.

The Examiner's answer to the evidence presented in Appellant's declaration under 37 C.F.R. §1.132, i.e., that the chemical treatments taught in Oliver I were lethal to eukaryotic cells, was to state that the data presented in Appellant's Declaration did not overcome the rejection,

> ". . . because the scope of comparative data presented does not commensurate [sic] with the scope of the claims. For example: the presented results are related to the effects of the prior art toxic substances and enzymes on viability of the T cell suspensions . . . Yet, the instant claims are not limited to the use of T cell suspensions. The comparatives [sic] data in the declaration are presented and/or argued for the cellular materials different from the prior art cellular materials. The cited prior art cellular materials are tissues including skin tissues that are within the scope of the instant claims." (Advisory Action issued 8/27/2004, at page 2.)

As evidenced by the above statement, it is clear that the Examiner has misunderstood the purpose for which Appellant conducted the experiments and presented the results described in the declaration. The data were not presented to support the scope of Appellant's claims; they were presented to demonstrate that the Examiner's interpretation of the Oliver I and II references was wrong as a matter of fact.

The Examiner has taken the data presented in the declaration as relating only to T cell suspensions, but this is incorrect: It is clearly stated in each experiment that murine splenocytes from both NOD and B6 mice, murine kidney cells, murine liver cells, and human peripheral blood lymphocytes were all treated according to Oliver I, Oliver II, or Stone in each of the experiments detailed in the declaration. (*See*, cell types listed under subheading "Protocol" on pages 4, 6, 8, and 9 of the declaration (Tab B).)

The Examiner has also argued that the declaration presents data that pertain to cellular materials that are different from the prior art cellular materials. But this is precisely Appellant's point: Appellant's invention relates to the use of viable tissue, and the prior art (Oliver I) relates to the use of non-viable fibrous tissue. The declaration presents data showing what the effect of following Oliver I would be if attempted with living tissue: the tissue dies and is thus rendered unsuitable for use in the invention of the appealed claims. Appellant's conclusion is that Oliver I cannot anticipate the invention of the appealed claims if by following the Oliver I teachings none of the steps specified in the appealed claims can be performed.

In view of the foregoing, Appellant respectfully asserts that it is clear that the Oliver I patent does not disclose the temporary removal of MHC Class I surface proteins specifically from viable mammalian donor tissue to facilitate successful transplantation and maintenance of the viable tissue in a host mammal. The Oliver I reference instead teaches a method whereby the treated fibrous tissues are, and must be, dead prior to transplantation, which method does not meet critical elements of the appealed Claims 1-3 and 5-8. Accordingly, the Oliver I reference is insufficient to anticipate any of the rejected claims under 35 U.S.C. §102(b), and the final rejection of Claims 1-3 and 5-8 over Oliver I should be reversed.

II. The Oliver II reference does not anticipate the invention of Claims 1-3 and 5-7, 9 and 12 under 35 U.S.C. §102(b)

For anticipation under 35 U.S.C. §102 by a printed publication, that publication must teach each and every element or aspect of the claimed invention. *See*, quotation from MPEP §2131 in Argument section I, *supra*.

Similar to the situation with the Oliver I reference, there are likewise elements set forth in Appellant's claims which are clearly not taught by the Oliver II publication, hence the rejection for anticipation is in error.

In fact, the only difference between the teaching of Oliver I and Oliver II is the replacement of the glutaraldehyde pre-treatment step (Oliver I) with a (supposedly) less toxic polyisocyante pre-treatment step (Oliver II):

> "It has been found unexpectedly that treatment of the preparation with polyisocyanates . . . can also result in the preparations having little or no propensity to be cytotoxic when implanted. This is in contrast to aldehyde treated preparations where residual cytotoxicity may be found." (See, column 2, lines 59-67.)

Appellant notes that there is a recognition in Oliver II that not only are the pre-treatments toxic to living tissue associated with the donor tissue, but the residual aldehydes associated with the donor tissue may be toxic to the host recipient.

The donor tissue treatments taught in Oliver II are almost identical to the treatments taught in Oliver I but for the polyisocyanate substitution for glutaraldehyde and an initial tissue treatment in Oliver II with acetone -- a chemical treatment which is also well known to be lethal to eukaryotic cells. These treatments, it is remembered, are in addition to a 28-day exposure to 0.5mg/ml sodium azide.

Therefore, the direct recitation in Appellant's Claim 1 of using "viable" donor tissue again immediately renders the teachings of Oliver II irrelevant and non-anticipatory to the present invention, as does the direct recitation in Claim 1 of treating the donor tissue to "temporarily ablate" MHC Class I antigens, preserving the ability of the donor tissues to re-express the MHC Class I antigens post-transplant. As in Oliver I, Oliver II cannot meet these features of Appellant's claims, because all references to antigenic

structures in Oliver II relate to their removal from the transplant tissue and none relate to reassertion of such structures. Since Oliver II does not meet recited features in Appellant's Claim 1, the rejection of Claim 1 as anticipated by Oliver II is in error and should be reversed.

Since appealed Claims 2-3, 5-7, 9, and 12 likewise incorporate the recitations of viable transplant tissue and the retained ability of the donor tissue for expressing the MHC Class I antigen complexes that were ablated prior to transplant, those claims also cannot be anticipated by Oliver II. Accordingly, their rejection as anticipated by Oliver II should likewise be reversed.

Elements of appealed Claims 5-7 and 12 additionally not met by Oliver II

As stated above for Oliver I, appealed Claims 5-7 are not anticipated by Oliver II because they specifically recite transplant tissues not contemplated by Oliver II.

Appealed Claim 12 recites the treatment of viable donor tissue to "temporarily ablate" MHC Class I surface antigens from the surface of the donor tissue preparatory to transplanting the tissue into a host. Claim 12 includes the additional step of "(d) transplanting a second donor tissue into the host." Such "serial grafts" are useful for the "pre-tolerization" of the host immune system to the second transplanted tissue. (*See*, for example, page 6, lines 6-12, of Appellant's specification.) In the final Office Action, the Examiner indicates that Oliver II discloses transplantation of a second donor tissue (hence the rejection of Claim 12 over Oliver II but not Oliver I). However, a review of Oliver II makes it apparent that no teaching or disclosure of the transplantation of a second donor tissue is present anywhere in Oliver II's specification or claims. Thus, Oliver II does not anticipate the method of Claim 12.

The Examiner's Arguments

The Examiner's answer to Appellant's point that the Oliver II reference relates to the use of non-viable tissue (as opposed to the viable tissue recited in Appellant's claims) was to argue that the treatment taught by Oliver II was not taught to be lethal to the tissue.

In other words, as with Oliver I, the Examiner challenged what Appellant asserted would be conveyed to a person of ordinary skill in the art, namely, that the Oliver II teaching was *designed* to eliminate viable tissue from the fibrous tissue selected in Oliver II for use in transplantation.

As discussed above, Appellant provided a declaration under 37 C.F.R. §1.132 in order to demonstrate to the Examiner that treatment of viable cells according to the Oliver I and II patents, e.g., in 0.5mg/ml sodium azide for 28 days, would, without question, result in 100% elimination of viable cells. As Oliver II teaches the identical sodium azide treatment taught in Oliver I, the data presented in Appellant's declaration with respect to sodium azide treatment discussed above are equally applicable with respect to Oliver II.

In addition, unlike the Oliver I reference, the Oliver II reference teaches several incubations of the donor tissue initially in acetone, in one example for up to 36 hours (*see*, Example 3) before the treatment in 0.5mg/ml sodium azide for 28 days.

The declaration under 37 C.F.R. §1.132 of Dr. Denise L. Faustman (Tab B) also includes a set of experiments showing the results of treatment of various human- and murine-derived eukaryotic cells for one hour in acetone. Specifically, Dr. Faustman treated murine liver cells, murine kidney cells, murine splenocytes, and also human peripheral blood lymphocytes (PBLs), in acetone for one hour, followed by washing in RPMI. Results of these treatments are shown in Tables 3A, 3B, and 3C included in the declaration. As seen in Tables 3A, 3B, and 3C, 100% of the various cells treated with acetone for one hour were non-viable within 22 hours after treatment.

These experiments graphically illustrate what is known, as a matter of law, by the hypothetical person of ordinary skill in the art, i.e., that the pre-transplant treatments of Oliver II, exactly as in Oliver I, render the donor tissue non-viable (regardless of whether such non-viability is discussed *in haec verba* in the Oliver II reference or not). This being the reality of the Oliver II patent teachings, the Oliver II patent cannot be considered to teach Appellant's invention, which calls for transplantation of viable tissue.

In view of the foregoing, Appellant respectfully asserts that it is clear that the Oliver II patent, like Oliver I, does not disclose the temporary removal of MHC Class I

surface proteins specifically from viable mammalian donor tissue to facilitate successful transplantation and maintenance of the viable tissue in a host mammal. The Oliver II reference instead teaches a method whereby the treated fibrous tissues are, and must be, dead prior to transplantation, which method does not meet critical elements of the appealed Claims 1-3, 5-7, 9, and 12. Accordingly, the Oliver II reference is insufficient to anticipate any of the rejected claims under 35 U.S.C. §102(b), and the final rejection of Claims 1-3, 5-7, 9, and 12 over Oliver II should be reversed.

III. The combination of Oliver I and Oliver II taken with Galati does not render the subject matter of Claims 1-9, 12-14 and 16-23 obvious under 35 U.S.C. §103(a)

In the final Office Action, the Examiner maintained the rejection of Claims 1-9, 12-14, and 16-23 under 35 U.S.C. §103(a) as being unpatentable over Oliver I and Oliver II, taken with Galati.

For the reasons set forth above, the teachings of Oliver I and Oliver II can be seen to be unrelated to the novel methods taught in Appellant's application and, as discussed below, the Galati reference does not provide a "bridge" linking the teachings of either Oliver I or Oliver II with the present invention.

The concept of attempting to prevent transplant rejection of viable donor tissue by a host by temporarily ablating the MHC Class I antigen complexes present on the surface of the donor tissue prior to transplantation, while maintaining the abililty of the donor tissue to re-express those ablated MHC Class I antigen complexes after transplant, is nowhere found in the cited prior art. As exhaustively reviewed above, Oliver I and Oliver II contain teachings relating only to the use of non-living, fibrous tissue which has been treated to ensure that no living cells are associated with it. The secondary Galati reference does not even relate to the field of transplantation. Rather, Galati discloses an *in vitro* method for MHC Class I antigen expression involving the preparatory step of removing intact MHC Class I antigen complexes from living cells, then collecting the complexes and measuring an acid-isolated component thereof (β_2-microglobulin) as a means of quantitating the expression level of Class I antigen complexes on the original

cells. No reference to transplantation of living cells is made in Galati; it is a document relating to laboratory methods.

Thus, the combined disclosures of Oliver I, Oliver II and Galati do not suggest any treatment of viable donor tissue for transplant that (a) removes MHC Class I antigen complexes temporarily but (b) leaves the ability of the donor tissues to express such MHC class I antigens intact. As disclosed in Appellant's application (page 6, lines 1-8), the ability of the donor tissue to continue to express the MHC Class I antigen complexes ablated prior to transplant is an important feature for inducing tolerance to the transplant, i.e., via the same internal mechanism (MHC Class I antigen presentation) that the host relies on for recognizing its own tissues as "self". These features are not suggested by the combination of Oliver I, Oliver II and Galati.

Elements of appealed Claims 5-7, 12-14 and 16-21 additionally not suggested by the combination of Oliver I and Oliver II taken with Galati

In addition to the reasons given above, the subject matter of appealed Claims 5-7, 12-14, and 16-21 cannot be suggested by the combination of Oliver I and II taken with Galati. Claims 5-7, as seen above, recite specific donor tissues expressly excluded from the Oliver I and II teachings. Claims 12-14 and 16-21 recite the particular step of "(d) transplanting a second donor tissue into said host animal." This second transplantation relates to the pre-tolerization embodiments of the present invention discussed, e.g., at page 6, lines 8-12, of the specification. A secondary transplantation is not suggested by Oliver I or II. Furthermore, since Galati does not relate at all to the field of transplantation and makes no mention of transplantation, Galati likewise cannot supplement Oliver I and II to provide a suggestion of the features recited in Claims 5-7, 12-14, and 16-21.

The combination of Oliver I and Oliver II, taken with Galati, does not suggest transplantation of the specific tissues and organs specified in Claims 5-7 and does not suggest the two-stage transplantation method of Claims 12-14 and 16-21. Accordingly, in addition to the failure of the reference combination to render obvious the use of viable

tissue in accordance with Claim 1and its dependent claims, the reference combination also fails to render obvious the particular embodiments of Claims 5-7, 12-14, and 16-21.

For the foregoing additional reasons, Claims 5-7, 12-14, and 16-21 are not obvious under 35 U.S.C. §103(a), and their final rejection under that section should be reversed.

The Examiner's Arguments

The Examiner's answer to Appellant's distinguishing remarks as to the effect of the Galati reference on the combination of Oliver I and Oliver II was to minimize the reliance placed on the secondary reference:

> "[A]pplicant argues that the reference by Galati does not mention a transplantation of papain treated tissue . . . However, this reference is relied upon to demonstrate that the papain enzyme removes antigenic structures that are the MHC Class I antigens as encompassed by the claimed invention. The transplantation of the papain treated donor tissues is taught by the cited patents [Oliver I] and [Oliver II] combined." (See, final Office Action, pages 11-12.)

Thus, the Galati reference is apparently relied on for its teaching that papain has the enzymatic activity to remove MHC Class I antigens from the surface of cells. This fact, however, is not in dispute: See, e.g., page 6, lines 25-29, of Appellant's specification. The final obviousness rejection, therefore, depends on the suggestions provided by Oliver I and II combined with the well-known fact that papain cleaves MHC Class I molecules.

In this regard, the Examiner continuously overlooks the primary distinguishing feature between the Oliver I and II documents and the claimed invention, namely, that the references relate to the preparation and use of non-viable tissue, whereas the claimed invention specifies the use of viable tissue. Furthermore, the references discuss only the removal of antigenic structures from the transplant tissue, whereas the Appellant's claims *require* that the ability to re-express the initially removed MHC Class I antigen complexes is preserved.

Appellant asserts that the combination of the Oliver I and Oliver II patents and Galati does not suggest a method for inhibiting transplant rejection by treating viable donor tissue to temporarily ablate MHC Class I surface antigens and then transplanting the treated, viable tissue into a host where it retains its viability, including the ability to re-express the MHC Class I surface antigens initially ablated. At most, the Oliver I and Oliver II references teach that a sterilized fibrous tissue completely stripped of all surface molecules and completely and permanently devoid of all cellular function is suitable as a transplantable scaffold for host cell infiltration leading to wound repair. As demonstrated above, that is what is disclosed and claimed in the Oliver I and Oliver II patents. The addition of Galati as a secondary reference disclosing papain's enzymatic properties does nothing to make the reference combination contain a suggestion of the claimed invention. Accordingly, the rejections based on the combination of Oliver I and Oliver II taken with Galati should be reversed.

IV. The combination of Oliver I and Oliver II taken with Galati and further in view of Stone does not render the subject matter of Claims 10 and 11 obvious under 35 U.S.C. §103(a) further in view of Stone

Claims 10 and 11 depend from Claim 8 (specifying treatment of the donor tissue with a second enzyme) which, in turn, depends from Claim 1 (specifying, *inter alia*, treatment of the donor tissue with an enzyme effective for temporary ablation of MHC Class I antigens from the tissue). Claim 10 specifies that the second enzyme is α-galactosidase; Claim 11 specifies that the donor tissue is treated with a combination of papain and α-galactosidase.

As demonstrated in detail above, the Oliver I and Oliver II patents, taken with the Galati reference, would not have suggested to a person of ordinary skill in the art at the time of Appellant's invention a method for inhibiting rejection of viable transplant tissue by temporarily ablating MHC Class I antigens from the surface of said viable donor tissue prior to transplantation. The inclusion of Stone as an additional reference does not improve this combination and in fact renders the combination *less* capable of suggesting

Appellant's invention, by providing additional teachings *away* from the use of viable donor tissue for transplantation.

With respect to Stone, the Examiner states,

> "[Stone] discloses a method for inhibiting transplant [sic, transplant rejection] wherein the method comprises [the] step of treating donor tissue with galactosidase and [the] step of transplanting the treated tissue into host recipient and wherein the method results in a reduction of inflammatory reaction or immune response of recipient host." (See, final Office Action, page 13.)

The Stone reference reports on the effect of eliminating α-Gal epitopes from porcine articular cartilage by incubation with α-galactosidase followed by implantation into the suprapatellar pouch of cynomolgus monkeys and monitoring of the immune response. (*See*, Stone, at page 1578, right column, 1st paragraph.)

According to Stone,

> "This study shows that treatment of cartilage with α-galactosidase can successfully prevent anti-Gal immune response against the xenograft. However, the primate immune system reacts against cartilage-specific antigens, resulting in antibody formation as well as macrophage-mediated chronic inflammatory reaction in some of the xenografts." (See, page 1578, right column, 1st paragraph.)

Stone teaches the use of cartilagenous tissue which clearly still exhibits porcine antigens that cause immune attack of the transplanted tissue by the host immune system. There is no teaching relating to the viability of the transplant tissue and no suggestion that the transplanted tissue retains the ability to re-express any temporarily removed MHC Class I surface antigens.

Indeed, as taught by the Stone reference, the harvested cartilage tissue is prepared for transplant by first immersing in alcohol for 5 minutes followed by immersion in a phosphate-citrate-sodium-chloride buffer containing 100 U/ml α-galactosidase for 4 hours at 26°C. (*See*, Stone, Materials and Methods, page 1578.) Appellant asserts that it would be appreciated by a person of ordinary skill in the art that such treatment with alcohol

would be lethal to the transplant tissue, and Appellant has presented proof of this fact in the declaration under 37 C.F.R. §1.132 of Dr. Denise L. Faustman, attached at Tab B.

In paragraphs 16-18 of the declaration, several types of donor tissues are subjected to treatments according to the teaching of Stone. Specifically, murine splenocytes, kidney cells, and liver cells, and human PBLs, were treated for 5 minutes with alcohol, treated with alcohol followed by incubation with 100 U/ml α-galactosidase in phosphate-citrate-sodium-chloride buffer for 4 hours at 26°C, treated with α-galactosidase only, or treated with buffer only (control). As seen in Tables 4A, 4B, and 4C of the declaration, in the two protocols that included alcohol treatment, 100% cell death resulted within four hours. Treatment of cells in α-galactosidase alone or buffer alone (i.e., no alcohol treatment) had no effect on tissue viability.

Accordingly, as with Oliver I and II, the Stone reference relates to the use of non-viable tissue for transplantation.

The reference combination of Oliver I and Oliver II, taken with Galati, further in view of Stone provides no guidance to a person of ordinary skill in the art to suggest the method of Appellant's claims, because to follow the combined teachings leads, first, to the selection of fibrous or cartilagenous tissues, then to pre-treatment with reagents including formaldehyde, glutaraledhyde, acetone, alcohol, and sodium azide, which is intended to kill any living tissue associated with the selected fibrous or cartilagenous tissue. Such pre-treatment is then followed by enzymatic treatments and sterilization prior to transplantation. There is no mention in any of the references, and no suggestion from the combined teachings of the references, that the ability of the transplant tissue to re-express MHC Class I antigens must be preserved, as required by Appellant's methods. For the foregoing reasons, Appellant's invention as recited in Claims 10 and 11 is not obvious in view of the combination of references asserted by the Examiner, and the final rejection of Claims 10 and 11 should be reversed by the Board.

CONCLUSION

Appellant respectfully submits that all rejections of the final Office Action should be reversed, for the following reasons:

- The rejections based on 35 U.S.C. §102(b) are incorrect, as the cited references do not teach each and every element of Appellant's claimed invention. In particular, both the Oliver I and Oliver II references teach a transplantation method whereby the donor tissue is subjected to a harsh chemical regimen that is lethal to any living tissue associated with the selected fibrous donor tissue, and this precludes the references from meeting the requirement of Appellant's claims to employ "viable donor tissue".
- The two reference combinations relied on by the Examiner to maintain rejections under 35 U.S.C. §103(a) fail to establish the obviousness of any of Claims 1-14 or 16-23 because:
    - The reference combinations make no suggestion with respect to transplantation of viable tissue, therefore the combined teachings are not relevant to the field of Appellant's invention,
    - The reference combinations do not suggest the concept of temporary ablation of an antigenic structure from donor tissue prior to transplantation, which ablated antigenic structure is to be re-expressed following transplantation, therefore the combined teachings fail to suggest a central requirement of Appellant's claims, and
    - The Oliver I, Oliver II, and Stone references being irrelevant to transplantation of living tissues, and the Galati reference not pertaining to transplantation at all, the reference combinations asserted by the Examiner cannot suggest the critical features of Claim 1 (and its dependent claims) and cannot suggest the particular features of Claims 5-7, 12-14, and 16-21.

Accordingly, for the reasons set forth herein, the final rejections applied against appealed Claims 1-14 and 16-23 as set forth in the final Office Action of September 24, 2003 are in error and should be reversed by this Board.

Respectfully submitted,

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CERTIFICATE OF MAILING

The undersigned hereby certifies that this paper is being deposited with the U.S. Postal Service as First Class mail under 37 C.F.R. §1.8, postage prepaid, in an envelope addressed to: Mail Stop Appeal Brief - Patent, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date indicated below:

Oct. 25, 2004
date

David G. O'Brien

CLAIMS APPENDIX -- APPEALED CLAIMS

1. A method for inhibiting rejection by a host mammal of donor tissue from another mammal which is transplanted into the host mammal, said method comprising
(a) treating viable donor tissue with an enzyme effective for temporarily ablating MHC Class I antigens from said donor tissue,
(b) transplanting said treated, viable donor tissue into said host mammal before MHC Class I antigens are re-expressed on the surface of said donor tissue, and
(c) maintaining said viable donor tissue in said host.

2. The method according to Claim 1, wherein said donor tissue is from a mammal that is the same species as said host mammal.

3. The method according to Claim 1, wherein said donor tissue is from a mammal that is of a different species than said host mammal.

4. The method according to Claim 1, wherein said host mammal is a human.

5. The method according to Claim 1, wherein said tissue comprises blood cells, neurons, hepatocytes, cardiac cells, genetically modified cells, skin cells, precursor cells, endothelial cells, fibroblasts, myoblasts, islets of Langerhans cells, or bone marrow cells.

6. The method according to Claim 1, wherein said tissue is an organ or part of an organ.

7. The method according to Claim 6, wherein said organ is selected from the group consisting of skin, kidney, heart, pancreas, brain, and liver.

8. The method according to Claim 1, wherein said donor tissue is additionally treated with a second enzyme effective to remove an antigenic surface structure from said donor tissue.

9. The method according to Claim 1, wherein said enzyme is papain.

10. The method according to Claim 8, wherein said second enzyme is α-galactosidase.

11. The method according to Claim 8, wherein said donor tissue is treated with a combination of papain and α-galactosidase.

12. A method for inhibiting rejection by a host mammal of donor tissue from another mammal which is transplanted into the host mammal, said method comprising:
(a) treating a first viable donor tissue with an enzyme effective for temporarily ablating MHC Class I antigens from said donor tissue,
(b) transplanting said treated, viable donor tissue into said host mammal before MHC Class I antigens are re-expressed on the surface of said donor tissue, and
(c) maintaining said viable donor tissue in said host mammal, and
(d) transplanting a second donor tissue into said host mammal.

13. The method according to Claim 12, wherein said first donor tissue is donor lymphocytes.

14. The method according to Claim 12, wherein said second donor tissue is also treated prior to transplantation with an enzyme effective for removing MHC Class I antigens from said tissue.

16. The method according to Claim 12, wherein said first and second donor tissue is from a mammal that is the same species as said host mammal.

17. The method according to Claim 12, wherein said first and second donor tissue is from a mammal that is of a different species than said host mammal.

18. The method according to Claim 12, wherein said host mammal is a human.

19. The method according to Claim 12, wherein said first and second donor tissue independently comprises blood cells, neurons, hepatocytes, cardiac cells, genetically modified cells, skin cells, precursor cells, endothelial cells, fibroblasts, myoblasts, islets of Langerhans cells, or bone marrow cells.

20. The method according to Claim 12, wherein said first and second donor tissue is an organ or part of an organ.

21. The method according to Claim 20, wherein said organ is selected from the group consisting of skin, kidney, heart, pancreas, brain, and liver.

22. The method according to Claim 1, wherein said donor tissue is treated with a solution of papain at 5-60 mg/ml for a period of 5 minutes to 24 hours.

23. The method according to Claim 22, wherein said solution contains 20-28 mg/ml papain and said tissue is treated for 30-120 minutes.

EVIDENCE APPENDIX

Statement Pursuant to 37 C.F.R. §41.37(c)(1)(ix)

The Declaration under 37 C.F.R. §1.132 submitted herewith was entered by the Examiner in the second Advisory Action, dated August 27, 2004.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Denise L. Faustman
Serial No.:	09/913,664
Filed:	August 17, 2001
Entitled:	METHOD FOR INHIBITING TRANSPLANT REJECTION

ART UNIT: 1651

EXAMINER: V. Afremova

Atty. Docket No.: DLF-002.1P US

FILE COPY

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

DECLARATION OF DENISE L. FAUSTMAN UNDER 37 C.F.R. §1.132

I, Denise L. Faustman, hereby declare and state that:

1. I am the inventor and owner of the subject matter claimed in the above-identified patent application, U.S. Serial No. 09/913,664.

2. I am an Associate Professor of Medicine at Harvard Medical School and Director of the Immunobiology Laboratory at the Massachusetts General Hospital (MGH), Charlestown, MA.

3. The present application is directed to an improved method for the transplantation of allogeneic or xenogeneic donor tissue into a host recipient. Specifically, a method is disclosed whereby viable (live) donor tissue is treated with at least one enzyme to temporarily remove surface antigens, particularly MHC Class I surface antigens, prior to the transplantation step without rendering the donor tissue non-viable. The removal of the surface antigens helps attenuate the immune response of the host and facilitates acceptance of the foreign donor tissue. Once the viable transplant tissue is established in the host, MHC Class I surface antigens are reexpressed on the surface of the cells of the

donor tissue and are recognized by the host organism as "self" antigens, thereby preventing attack of the donor tissue by the immune system of the host. In a preferred method, the donor tissue is treated with either papain alone or a combination of papain and α-galactosidase.

4. I have been informed by my attorney, and it is my understanding, that the claims of my application have been rejected as lacking novelty in view of the teachings of two U.S. patents, namely, U.S. 4,399,123 to Oliver et al. (the '123 patent) and U.S. 5,397,353 also to Oliver et al. (the '353 patent), or as being obvious in view of the teachings of those Oliver et al. patents taken together with other references, namely, Galati et al. (Cytometry, 27:77-83, 1997) and Stone et al. (Transplantation, 65(12):1577-83, June 1998). I am familiar with these publications, having studied them in connection helping my attorneys answer an earlier office action issued in my application.

5. These references do not teach my invention or make it obvious because the two Oliver et al. patents (the '123 patent and the '353 patent) and also the Stone et al. article teach a treatment for donor tissue intended for transpant that kills all living cells associated with that tissue (and thus the donor tissue is not viable at the time of transplant). Furthermore, the Galati et al. article does not relate to preparation of cells for transplantation but rather to a method for obtaining MHC Class I antigen complexes removed from cells by papain digestion, as a means of quantitatively measuring the amount of MHC Class I molecules expressed.

6. I have been informed and believe that the Examiner has maintained the rejections based on the teachings of these documents, and I have been informed and believe that the Examiner has argued that the treatments of donor tissue taught in the '123 patent, the '353 patent, and the Stone et al. article are not disclosed to be lethal to donor tissues.

7. I am making this declaration to demonstrate that the treatments of donor tissues as described in the '123 patent, the '353 patent, and the Stone et al. article directly cause the tissue to quickly become non-viable and therefore unsuitable for use in my invention.

8. I personally conducted experiments following the teachings of the '123 patent, the '353 patent, and the Stone et al. article to treat murine and human eukaryotic cells with varying concentrations of the agents taught in those references. Specifically, I treated murine T cells isolated from splenocytes, kidney cells, and liver cells, or human peripheral blood lymphocytes (PBLs) with varying concentrations of sodium azide (as taught in both the '123 patent and the '353 patent), or with formaldehyde (taught in the '123 patent), or with acetone (taught in the '353 patent), or with alcohol + α-galactosidase (as taught in the Stone et al. document). The data are presented herein.

9. **I. Treatment of donor cells with sodium azide**

Example 1 of the '123 patent specifies that tissue intended for transplantation (human dermis) is treated with trypsin (2 mg/ml in 0.1M phosphate buffer) and 0.5 mg/ml sodium azide at 15° C for 28 days, prior to glutaraldehyde treatment (16 hours) and implantation into a host. Example 2 of the '123 patent specifies the same treatment, substituting chymotrypsin for the trypsin. Example 3 of the '123 patent specifies the same treatment, substituting rat tendon tissue for the human dermis of Example 1. Example 4 of the '123 patent specifies the same treatment using pig ligament in place of human dermis tissue. Example 6 of the '123 patent specifies the same treatment using pig dermis.

Example 1 of the '353 patent specifies treatment of pig dermis intended for transplant by immersion in acetone (1 hour), followed by washing in 0.1M phosphate buffer, then treatment with trypsin (2 mg/ml in 0.1M phosphate buffer) and 0.5 mg/ml sodium azide at 15° C for 28 days. Example 2 of the '353 patent specifies treatment of pig dermis intended for transplant by immersion in acetone for 2 hours, followed by washing in 0.1M phosphate buffer, then treatment with papain (3 mg/ml in 0.1M phosphate buffer), cysteine (0.01M), and 0.5 mg/ml sodium azide for 28 days. Example 3 of the '353 patent specifies treatment of pig dermis intended for transplant by immersion in acetone (39 hours), followed by washing in 0.1M phosphate buffer containing sodium azide (0.5 mg/ml) and trypsin (50 g/l) for 7 days, then dehaired and treated with the trypsin/sodium azide buffer for an additional 21 days.

Thus, the '123 and '353 patents both teach a protocol for treating donor tissue prior to transplant that requires contact of the tissue with sodium azide at a concentration of 0.5 mg/ml for about 28 days.

The '123 and '353 patents state that the sodium azide is included as a bactericide, however I followed the '123 and '353 patent teachings in order to demonstrate that the sodium azide treatment also results in the production of non-viable donor tissue for the transplant:

Protocol: Fresh murine T cells isolated from NOD and B6 mouse splenocytes, kidney cells and liver cells of B6 mice, and human peripheral blood lymphocytes (PBLs) were isolated and plated into 24-well plates at a density of 1×10^6 cells per ml.

Cells were incubated with sodium azide (NaN_3) at a particular concentration at 15° C. NaN_3 concentrations of 0 mg/ml, 0.001 mg/ml, 0.002 mg/ml, 0.01 mg/ml, 0.02 mg/ml, 0.1 mg/ml, and 0.5 mg/ml were tested. The cells were suspended in standard tissue culture media (RPMI with 10% FCS). The % viability of the cells as a function of sodium azide concentration was assessed after 22 hours and after 3 days incubation by flow cytometry using propidium iodide. Results of these experiments are shown in Tables 1A, 1B, and 1C.

Results: Table 1A shows the % viability of murine NOD and B6 T cells isolated from splenocytes and treated with varying concentrations of sodium azide. Table 1B shows the % viability of murine B6 T cells isolated from kidney and liver cells and treated with varying concentrations of sodium azide. Table 1C shows the % viability of human peripheral blood lymphocytes treated with varying concentrations of sodium azide.

Table 1A:
Sodium azide toxicity on T cells isolated from splenocytes of NOD and B6 mice

NaN_3 Concentration (mg/ml)	% viable cells (NOD/B6) at 22 hours	% viable cells (NOD/B6) at 3 days
0	75/80	65/72
0.001	55/65	0/0
0.002	50/61	0/0
0.01	50/55	0/0
0.02	21/35	0/0
0.10	5/5	0/0
0.50	4/2	1/0

Table 1B:

Sodium azide toxicity on liver and kidney cells from B6 mice

NaN_3 Concentration (mg/ml)	% viable cells (liver/kidney) at 22 hours	% viable cells (liver/kidney) at 3 days
0	85/82	81/82
0.001	20/14	2/5
0.002	20/15	1/0
0.01	5/4	0/0
0.02	1/2	0/0
0.10	0/0	0/0
0.50	0/0	0/0

Table 1C:

Sodium azide toxicity on human PBLs

NaN_3 Concentration (mg/ml)	% viable PBLs at 22 hours	% viable PBLs at 3 days
0	92	87
0.001	12	2
0.002	8	2
0.01	7	2
0.02	7	0
0.10	5	0
0.50	0	0

10. My experiments show that sodium azide treatment was very toxic to both freshly isolated murine and human cells at all the test concentrations of 0.001–0.5 mg/ml. After 3 days of exposure to sodium azide, all donor cells exposed to sodium azide concentrations above 0.01 mg/ml were uniformly and completely non-viable. All sodium azide experiments were terminated after 3 days as less than 5% viable cells remained in the tissue culture wells at all sodium azide concentrations (except control cells: 0 mg/ml sodium azide).

11. **II. Treatment of donor cells with formaldehyde**

The '123 patent teaches a method for preparing fibrous tissue for transplantation which includes two enzymatic treatments (proteolytic and carbohydrate-splitting enzymes), followed by contact with a crosslinking agent, i.e., glutaraldehyde or formaldehyde at a concentration greater than 0.01% (see, col. 3 (lines 45-65); col. 4 (lines 49-65) of the '123 patent). The '123 patent further specifies that treated tissue is only suitable for transplant "after its sterilization" (see, col. 3 (line 43 and line 48); col. 4 (line 53); col. 5

(line 2 and lines 26-30) of the '123 patent). Example 5 of the '123 patent specifies that samples of pig dermal collagen were treated with 0.1%, 1% or 5% formaldehyde for 21 days.

The '123 patent states that the formaldehyde treatment is to remove antigenicity of the treated tissue by crosslinking amino groups in the tissue (see, col. 3 (line 60) to col. 4 (line 7)); however, I followed the '123 patent teachings with respect to formaldehyde treatment in order to demonstrate that exposure to such crosslinking agents as glutaraldehyde or formaldehyde also results in the production of non-viable donor tissue for the transplant:

Protocol: Fresh murine T cells isolated from NOD and B6 mouse splenocytes, kidney cells and liver cells of B6 mice, and human peripheral blood lymphocytes (PBLs) were isolated and plated into 24-well plates at a density of 1×10^6 cells per ml.

The cells were treated for 5 minutes in either 0.1%, 0.2%, or 5% formaldehyde. The % viability of the cells as a function of formaldehyde concentration was assessed after 22 hours and after 3 days by flow cytometry using propidium iodide. Results of these experiments are shown in Tables 2A, 2B, and 2C.

Results: Table 2A shows the % viability of T cells isolated from murine NOD and B6 splenocytes 22 hours and 3 days after a 5 minute treatment with formaldehyde. Table 2B shows the % viability of murine B6 kidney and liver cells 22 hours and 3 days after a 5 minute treatment with formaldehyde. Table 2C shows the % viability of human peripheral blood lymphocytes 22 hours and 3 days after a 5 minute treatment with formaldehyde

Table 2A:

Formaldehyde toxicity in T cells isolated from splenocytes of NOD and B6 mice

Formaldehyde Concentration (%)	% viable cells (NOD/B6) at 22 hours	% viable cells (NOD/B6) at 3 days
0	78/86	70/78
0.1	11/15	0/0
0.2	8/14	0/0
5	0/0	0/0

Table 2B:

Formaldehyde toxicity in T cells isolated from liver and kidney cells of B6 mice

Formaldehyde Concentration (%)	% viable cells (liver/kidney) at 22 hours	% viable cells (liver/kidney) at 3 days
0	91/95	88/90
0.1	2/3	0/0
0.2	0	0/0
5	0	0/0

Table 2C:

Formaldehyde toxicity in fresh human PBLs

Formaldehyde Concentration (%)	% viable PBLs at 22 hours	% viable PBLs at 3 days
0	95	90
0.1	5	0
0.2	4	0
5	0	0

12. My experiments show that donor cells treated for only five minutes (as compared with 16 hours glutaraldehyde treatment (Examples 1-4 and 6) or 21 days formaldehyde treatment (Example 5) in the '123 patent) results in completely non-viable tissue in all cases within 3 days at concentrations of 0.1% or greater.

13. **III. Treatment of donor cells with acetone**

The '353 patent teaches a wet preparation of tissue intended for transplant involving initial extraction of the tissue with an organic solvent such as acetone (see, col. 4 (line 50) of the '353 patent) before sterilization with gamma radiation or hydrogen peroxide. Example 1 of the '353 patent specifies treatment of pig dermis intended for transplant by immersion in acetone for 1 hour, followed by washing and enzyme treatment. Example 2 of the '353 patent specifies treatment of pig dermis intended for transplant by immersion in acetone for 2 hours, followed by washing and enzyme treatment. Example 3 of the '353 patent specifies treatment of pig dermis intended for transplant by immersion in acetone for 39 hours, followed by washing and enzyme treatment.

The '353 patent thus teaches the use of acetone in every one of its examples of tissue preparation. I followed the '353 patent teachings with respect to acetone treatment in

order to demonstrate that exposure to such agents as acetone also results in the production of non-viable donor tissue for the transplant:

Protocol: Fresh murine T cells isolated from NOD and B6 mouse splenocytes, kidney cells and liver cells of B6 mice, and human peripheral blood lymphocytes (PBLs) were isolated and plated into 24-well plates at a density of 1×10^6 cells per ml. All cells were incubated with acetone for 1 hour and rinsed once in RPMI. The % viability of the cells as a function of the presence or absence of acetone was assessed after 22 hours by flow cytometry using propidium iodide. Results of these experiments are shown in Tables 3A, 3B, and 3C.

Results: Table 3A shows the % viability of T cells isolated from murine NOD and B6 splenocytes 22 hours after incubation for 1 hour with or without acetone. Table 3B shows % viability of murine B6 kidney and liver cells 22 hours after incubation for 1 hour with or without acetone. Table 3C shows % viability of human peripheral blood lymphocytes 22 hours after incubation for 1 hour with or without acetone.

Table 3A:

Acetone toxicity (1 hr) in T cells isolated from splenocytes of NOD and B6 mice

1-hour Acetone Exposure	% viable cells (NOD/B6) after 22 hours
no (control)	72/81
yes	0/0

Table 3B:

Acetone toxicity (1 hr) in liver and kidney cells of B6 mice

1-hour Acetone Exposure	% viable cells (liver/kidney) after 22 hours
no (control)	79/89
yes	0/0

Table 3C:

Acetone exposure (1 hr) on fresh human PBLs

1-hour Acetone Exposure	% viable cells (PBLs) after 22 hours
no (control)	93
yes	0

14. My experiments show that donor cells treated for one hour (cf. Example 1 of the '353 patent) results in completely non-viable tissue in all cases within 22 hours after acetone extraction.

15. The foregoing experiments show that following the teachings of the two patents of Oliver et al. (the '123 and the '353 patent) with respect to preparation of tissue for transplant results in non-viable tissue, which is not suitable for use in accordance with my invention.

16. **IV. Treatment of donor cells with alcohol + α-galactosidase**

The Stone et al. article teaches a method for preparing porcine articular cartilage for transplant. Cartilage plugs were immersed in alcohol for 5 minutes, then immersed in a phosphate-citrate-sodium chloride buffer containing 100 U/ml of α-galactosidase and incubated for 4 hours at 26° C. (See, Stone et al., page 1578, right column.)

The Stone et al. article states that the alcohol treatment of the donor tissue is to remove synovial fluid and lipid-soluble contaminants (page 1578, right column); however, I followed the Stone et al. teachings with respect to alcohol treatment in order to demonstrate that exposure to such reagents also results in the production of non-viable donor tissue for the transplant:

Protocol: Fresh murine T cells isolated from NOD and B6 mouse splenocytes, kidney cells and liver cells of B6 mice, and human peripheral blood lymphocytes (PBLs) were isolated and plated into 24-well plates at a density of 1×10^6 cells per ml. As in the Stone et al. article, the cells were exposed to alcohol for 5 minutes, washed and then incubated with 100 U/ml of α-galactosidase in phosphate-citrate-sodium chloride buffer for 4 hours at 26° C. Comparative wells of cells were also incubated in alcohol alone (followed by buffer only wash) or in the α-galactosidase buffer alone. The % viability was determined using flow cytometry after the 4-hour incubation. Results of these experiments are shown in Tables 4A, 4B, and 4C.

Results: Table 4A shows the % viability of T cells isolated from murine NOD and B6 splenocytes after 4 hours of incubation at 26° C with either alcohol alone, α-

galactosidase alone, or a combination of alcohol + α-galactosidase. Table 4B shows the % viability of murine B6 kidney and liver cells after 4 hours incubation at 26° C with either alcohol alone, α-galactosidase alone, or a combination of alcohol + α-galactosidase. Table 4C shows the % viability of human peripheral blood lymphocytes after incubation for 4 hours at 26° C with alcohol alone, α-galactosidase alone, or a combination of alcohol + α-galactosidase.

Table 4A:

Alcohol + α-galactosidase toxicity in T cells isolated from NOD and B6 splenocytes

Treatment	% viable cells (NOD/B6) after 4 hours
buffer only	92/94
alcohol alone (then buffer)	0/0
α-galactosidase buffer alone	89/91
alcohol + α-galactosidase	0/0

Table 4B:

Alcohol + α-galactosidase toxicity in B6 liver and kidney cells

Treatment	% viable cells (liver/kidney) after 4 hours
buffer only	98/91
alcohol alone (then buffer)	0/0
α-galactosidase buffer alone	95/90
alcohol + α-galactosidase	0/0

Table 4C:

Alcohol + α-galactosidase toxicity in human PBLs

Treatment	% viable cells (NOD/B6) after 4 hours
buffer only	98
alcohol alone (then buffer)	0
α-galactosidase buffer alone	95
alcohol + α-galactosidase	0

17. My experiments show that treatment of donor tissue for only five minutes with alcohol (as described in Stone et al.) results in non-viable tissue in all cases within four hours after alcohol treatment. Exposure of tissues to α-galactosidase alone does not appear to be toxic to donor tissues.

18. The foregoing experiments show that following the teachings of the Stone et al. article with respect to preparation of tissue for transplant results in non-viable tissue, which is not suitable for use in accordance with my invention.

19. Conclusions

(a) As seen in Tables 1A-1C, treatment of viable tissues with sodium azide at concentrations as low as 0.001mg/ml (that is, 500 times less concentrated than taught by Oliver et al.) results in the production of 100% non-viable tissue. The absence of any viable cells was observed as early as 22 hours post-treatment, and all treated cells were non-viable by 3 days post-treatment (that is, within a period 24 days shorter than taught by Oliver et al.).

(b) As seen in Tables 2A-2C, treatment of viable tissues with an aldehyde cross-linking agent such as formaldehyde for as little as 5 minutes (as compared with incubation for hours or weeks taught by Oliver et al.) results in the production of 100% non-viable tissue after incubation in concentrations as low as 0.1%. The absence of any viable cells was observed as early as 22 hours post-treatment, and all treated cells were non-viable by 3 days post-treatment.

(c) As seen in Tables 3A-3C, treatment of viable tissue with acetone for 1 hour (as taught in the '353 patent) results in 100% non-viable tissue within 22 hours post-treatment.

(d) As seen in Tables 4A-4B, treatment of viable tissues with alcohol for as little as 5 minutes results in 100% non-viable tissue within 4 hours post-treatment.

20. The foregoing experiments confirm that the teachings of the '123 patent, the '353 patent, and the Stone et al. article teach treatments for intended transplant tissue which render the tissue non-viable almost immediately. In contrast to this, my invention requires that the pre-tranplant treatment of viable donor tissue leaves the tissue viable, and that the viability is maintained after transplant. Thus, the Oliver et al. patents do not teach my invention. Moreover, combining the teachings of the Oliver et al. patents with the Galati et al. and the Stone et al. articles still results in the teaching of a pre-treatment for donor tissue that leads to non-viable tissue prior to transplant, and therefore that combination of publications cannot render my invention obvious.

21. I further declare that all statements made herein of my own knowledge are true and that statements made on information and belief are believed to be true and further that false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

3/16/04
date

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